Detailed Methods

Microarray Fabrication

The microarrays used for the experiments contained 17,600 human cDNA clones and were prepared from two different clone sets including *Incyte* UniGEM2 set (Fremont, CA**)** and *Research Genetics* Named Genes set (Research Genetics, Huntsville, AL**)**. These cDNA clones are enriched for known genes and can be classified into several groups based on their biological functions, such as stress proteins, cell cycle control, signal transduction, apoptosis, transcription factors, DNA repair and replication, cytokines, etc. The cDNA clones from *Research Genetics* were inoculated in LB medium containing 100µg/ml cabenicillin (*Sigma*, St. Louis, MO). The plasmid templates were isolated using *Promega* Wizard SV 96 plasmid DNA purification system (Madison, WI). Then, the plasmid templates were PCR amplified using *Qiagen* HotstarTaq Master Mix Kit (Valencia, CA) with GF200R/F primers (*Research Genetics*, Huntsville, AL). The amplified PCR products were purified using Centri Sep 96 filter plates (Princeton Separations, Adelphia, NJ). The purified PCR products were vacuum-dried and resuspended in 3X SSC (0.45M NaCl, 0.045M sodium citrate). PCR products of UniGEM2 set were purchased directly from *Incyte* (Fremont, CA). All 17K cDNAs were spotted onto poly-L-lysine-coated slides (NCI ROSP 17K Human Array) according to Eisen & Brown (Eisen and Brown, 1999) using an OmniGrid arrayer (GeneMachines, San Carlos, CA).

RNA Extraction

For each collection point, the cell monolayer was washed once with PBS (4°C). The cells (~15 x 10^6) were then scraped in 10 ml PBS (4°C) and centrifuged (1000 rpm at

4°C, 5 min**)**. Briefly, 1 ml of Trizol reagent (Life Technologies, Gaithersburg, MD) was added to the cell pellets, vortexed, and incubated for 5 min at room temperature. The mixture was transferred into 1.5 ml centrifuge tubes and 200 µl of chloroform per ml of Trizol was added to partition the phases. The mixture was centrifuged (10,000 rpm, room temperature**)** for 10 min. After centrifugation, the aqueous layer was transferred into a new 1.5 ml centrifuge tube, and a 1/2 volume of 2-propanol was added to the samples. Samples were stored at –80°C until probe preparation. Samples were thawed on ice and pelleted by centrifugation (14,000 rpm**)** for 20 min at 4°C and washed twice with 1 ml of 70% ethanol. The pelleted RNA was resuspended in 180 µl of DEPC water and 20 µl 10X DNase reaction buffer (Ambion, Austin, TX) was added. After DNase treatment (5 µl Ambion RNase free Dnase, Austin, TX) for 10 min at 37°C, RNA was re-precipitated by addition of 1/10 volume of 3M ammonium acetate (pH 5.2) followed by 2 volumes of 100% ethanol and, centrifuged (14,000 rpm, at 4°C, 20 min**)**, and the pellet resuspended in DEPC water, and absorbance at 260 and 280 nm were measured in order to determine both concentration and quality. Then, 100 µg of purified RNA was further purified using Qiagen Rneasy Mini Kit (Valencia, CA) according to manufacturer's instructions.

Probe Labeling and Microarray Hybridization

The methods for probe labeling reaction and microarray hybridization were used as described previously (Khan et al., 1998) with a few modifications. For all experiments, the cDNA probes from untreated and treated MCF7 cells were compared against a reference probe that was generated from a universal human reference RNA (Stratagene, La Jolla, CA), which consisted of RNAs isolated from 10 different cell lines. The

advantage of using a universal reference probe is to minimize variation of plating effects and cell progression for time course experiments. 40 µg of MCF-7 RNA or 20 µg universal reference RNA was labeled with Cy5 and Cy3 respectively by using Superscript II Reverse Transcriptase (Invitrogen, Carlsbad, CA). Briefly, after heating the RNA and primer to 60°C for 5 min, the samples were placed at 42°C and the 5x buffer, DTT, RNasin (Promega; Madison, WI), SSII and DNTP's (Amersham-Pharmacia; Piscataway, NJ) was added. Buffer, DTT and enzyme were supplied in the Superscript II Reverse Transcriptase kit. DTTP was reduced to 4 mM in the standard 10 mM DNTP mix and 4 µl of Cy3 or Cy5 dUTP (NEN Life Sciences, Boston, MA) was added to the reaction tube. The samples were incubated at 42°C for 50 min. The reaction was stopped with 5 µl of 0.5M EDTA and heated to 65°C. 1 N NaOH was added to hydrolize the residual RNA and the samples were incubated at 65°C for 1 hr. Samples were purified in Bio-6 Chromatograph column (Bio-Rad, Hercules, CA) and concentrated with Microcon 30 membranes (Millipore Corp., Bedford, MA) to 17 µl.

The arrays were pre-hybridized with buffer (5X SSC, 0.1% SDS, 1% BSA) at 42°C for 1 hr. Slides were washed in de-ionized water followed by 2-propanol. Cy5- and Cy3-labeled cDNA samples were mixed with 1 µl COT1-DNA (10 µg/µl) (Invitrogen, Carlsbad, CA), polyA (8-10 µg/µl) (Amersham Pharmacia Biotech, Piscataway, NJ), and yeast tRNA (4 µg/µl) (Ambion, Austin, TX) for hybridization. The mixed samples were denatured and after the addition of 20 µl of 2x hybridization buffer (50% formamide, 10X SSC, 0.2% SDS) the entire sample was loaded onto the slides for overnight hybridization at 42°C. Following hybridization, the hybridized slides were then washed

in 2x SSC, 0.1%, 1X SSC, 0.1% SDS, and 0.2x SSC for 4 min each followed by a 1 min wash in 0.05x SSC. Slides were then placed in 2-propanol followed by spin dry.

TaqMan Assay

After initial expression analysis, 11 clones were selected based on the following criteria: 1) > 2 fold induction; 2) high correlation among the three replicates; and 3) signal intensities > 4000 for both channels. In addition, one clone (GADPH) was selected as a control gene. Quantitative RT-PCR was performed using 1 μg of total RNA and 1 μg of random heximer primer in 10 μl reaction at 70°C for 10 min, then the primed RNA was incubated in a 25μl reaction mixture with 1X First-Strand Buffer, 10 mM DTT, 0.25 mM dNTPs, 200U Superscript II (Invitrogen, Carlsbad, CA) at 42°C for 1 hr and then at 70°C for 10 min to inactive Superscript II. After RT, all samples were diluted 1:9 with sterile water and 4 μl was used for each SYBR Green PCR assay. Primer sequences were designed using Primer Express software (Applied Biosystems, Foster City, CA). The primer sets were selected to amplify the sequences as close as possible to the 3' coding region of the target genes. The sequences of IMAGE clones used in this study can be found in the GenBank database.

Real-time PCR was performed using the ABI Prism 7900HT sequence detection system according to manufacturer's instructions. Briefly, PCR premixes contained all reagents (1X SYBR Green PCR master mixture, 100 pM each forward and reverse primer in a final volume of 10 μl) except templates were prepared and aliquoted into 384 well reaction plates. The cDNA templates were added last and thermal cycling was performed with a hot-start at 95ºC for 10 min and then 40 cycles of reactions (15 sec at 95ºC and 1 min at 60ºC). Dissociation curve analysis of amplification products was performed at the

end of each PCR reaction by cooling the samples to 60ºC and then increasing the temperature to 95ºC at 0.2 ºC /sec. Amplicons were approximate 50-150 bps long. All SYBR Green PCR results were analyzed by SDS sequence detector 2.0 software (Applied Biosystems, Foster City, CA). Direct detection of PCR products was monitored by measuring the increase in fluorescence from the binding of SYBR Green I dye to double-stranded DNA. These measurements resulted in an amplification plot of the fluorescence signal vs. cycle number. The parameter Ct (threshold cycle) was defined as the fractional cycle number at which the fluorescence exceeds the fixed threshold. Quantitation of the target in unknown samples was accomplished by measuring Ct. Moreover, all PCR products were electrophoresed to verify their sizes.